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EFFECT OF FOLIAR APPLICATION OF BIOSTIMULANTS ON FORAGE YIELD IN ALFALFA *(Medicago sativa* L.*)*

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ABSTRACT

The present study was aimed to determine the effect of V_2, VR_{10} and VR_1 experimental biostimulants application on yield related main traits (plant height and stems number per m^2) and on forage productivity of Prista 4 alfalfa variety. The study was carried out in experimental field at Institute of Agriculture and Seed Science "Obraztsov chiflik" - Ruse, from 2019 to 2021. The biostimulants were foliarly applied two times in each regrowth. There was found positive effect of the biostimulants application on the growth and development of alfalfa. The additional introducing of VR_{10} and VR_1 biostimulants contributed to higher alfalfa grass stands by 17.13 and 14.36%, respectively compared to control. Foliar spraying with V_2 biostimulant had more clear pronounced positive effect on the stem development. According to the three-year results the average stems number per m^2 at V_2 treatment significant exceeded the control by 4.78%. V_2 biostimulant distinguished with a stronger positive effect on the forage productivity among studied products. Its application resulted in significant increases on green mass and dry matter yields by 4.18 and 4.99%, respectively over the control. V_2 application gives promising results and reason to be recommended as a corrective component in technology of growing alfalfa for forage.

Keywords: Alfalfa, biostimulants, plant height, stems number, yield.

INTRODUCTION

The satisfaction of the population plant and animal productions needs poses a serious challenge to agriculture (Santini et al., 2021). A high yield per unit area obtaining is based on the cultivation of varieties with high genetic potential using appropriate agronomic practices.

Inorganic products (synthetic fertilizers and pesticides) application is an effective method to increase yields, but is costly (30 to 50% of production costs, depending on the crop) and also leads to environmental problems (Kopittke et al., 2019; Zambrano-Mendoza et al. 2021).

Innovative environmentally friendly practices, considering aspects of soil and groundwater health and biodiversity conservation, are the reduction of the use of chemical products and application of technologies for organic production (Mehmed and Enchev, 2020).

The biobased products such as organic foliar fertilizers, growth regulators and biostimulants represent a sustainable, efficient technology or complement to their synthetic counterparts (i.e., agrochemicals) to improve nutrient use efficiency and secure yields stability of agriculture under optimal and stressful conditions (Bozhanska et al., 2017; Mihova et al., 2017; Stoyanova et al., 2021).

The foliar fertilization has been the subject of many field studies and has become widely adopted as a standard practice for many crops in a lot of countries (Ozturk and Yildirim, 2013; Wani et al., 2017; Ivanova-Kovacheva et al., 2018; Ivanov and Nikolov, 2021).

The alfalfa (*Medicago sativa* L.), also known as “Queen of Forage” is the most important perennial forage legume widely grown throughout the world because its high yield, quality, nutritional value and adaptability (Bouton, 2012). In Europe alfalfa is considered to be the species with the greatest contribution to sustainability of the agriculture (Huyghe, 2003). The yield increasing and improving the quality of alfalfa is of great importance for protein problem deciding under now existing market relations. A number of studies are related to search opportunities for increasing alfalfa forage yield by applying different agrotechnical practices - optimal density; intensity of utilization; intercropping, balanced fertilization (Sevov et al., 2007; Ceylan et al., 2009; Petkovic et al., 2019; Basaran et al., 2020).

The fertilization of alfalfa has been traditionally focused on effects of direct application of phosphorus (P) and/or potassium (K) in various combinations (Berg et al., 2007, Macolino et al., 2013). There is a little data available, indicating that integrated alfalfa nutrition involving the combined use of inorganic and organic fertilizers increases yield more than they application alone (Quansah, 2000). Vasileva and Kostov (2015) have reported the alfalfa yield was more stable under organic fertilization conditions as compared with mineral ones.

The present study was aimed to determine the effect of V_2, VR_{10} and VR_1 experimental biostimulants application, an elaboration of the Institute of Cryobiology and Food Technologies (ICFT) - Sofia, on yield related main traits (plant height and stems number per unit area) and on forage productivity of alfalfa.

MATERIAL AND METHODS

The study was carried out in experimental field at Institute of Agriculture and Seed Science (IASS) "Obraztsov chiflik" – Ruse, Agricultural Academy, Bulgaria from 2019 to 2021. The soil type of experimental site was leached black soil, located on sandy clay. Active soil fertility was characterized by good potassium (33.17 mg 100 g^{-1} soil), insufficient nitrogen (16.84 mg 100 g^{-1} soil) and poor phosphorus (6.15 mg 100g^{-1} soil) nutrient regime. The humus content was low and ranged from 2.03% to 2.17% (for the layer from 0 to 40 cm). The soil reaction was slightly acid (pH - from 5.84 to 5.94).

Data for monthly temperature sums and rainfall during the three growing seasons are presented in Table 1. Long-term norm (LTN) represents average value of collected data about monthly temperatures and precipitation in the period 1895-2005. There was a considerable variability in rainfall amounts and its distribution during years of study. The year of alfalfa sowing characterized by a high temperatures and sufficient soil moisture in March. The precipitation rate in April and May of first year of the experiment was above LTN by 25.9 mm and 32.2 mm, respectively. There were prolonged rainy periods in June and August. The monthly temperature sums were close to LTA, with higher temperatures in April.

Table 1. Monthly temperature sums and monthly precipitation from 2019 to 2021

Months	Monthly rainfull, mm				Σ T °C			
	2019	**2020**	**2021**	**LTN***	**2019**	**2020**	**2021**	**LTN***
Σ October- March	327.2	259.7	320.3	235,1	1191.2	758.9	837.6	
April	76.6	75.2	8.6	50.7	437.5	345.4	483.7	341.8
May	98.3	90.3	37.1	66.1	493.2	506.2	605.6	512.2
June	74.2	62.1	105.4	80.5	660.7	655.2	657.8	606.6
July	2.2	84.7	167.8	67.4	762.3	727.4	692.6	697.7
August	54.1	34.0	13.3	49.4	708.8	735.9	739.4	739.8
September	16.7	42.2	65.8	44.6	583.0	586.0	570.6	535.3
Σ April-September	322.1	388.5	398.0	358.7	3556.1	3507.6	3749.7	3426.2

Legend: * LTN (Long term norm) - the period from 1896 to 2006 was used

The second year of the experiment (2020) was distinguished by the monthly temperature sums close to LTN, and with slightly higher temperatures in June and July. Regarding the precipitation, except for June and August, during other months, its amount was higher than the LTN.

There were conditions of prolonged drought with higher temperatures and precipitation amount significantly below the relevant LTNs during April and May in the third year. From June to July, monthly temperature sums were close to LTN and the precipitation rate was significantly above LTN.

The investigation was conducted with Prista 4 alfalfa variety, without irrigation. Accorging fall dormancy (FD) scored scale Prista 4 variety is moderately dormant alfalfa (FD rating 5). The study was designed in a randomized block design in 4 replicates, at a individual plot size of 5 m^2. Sowing was performed by machine at a inter-row spacing of 12.5 cm with the seeding rate of 2.5 kg da^{-1}. The field trial included a control (without foliar fertilization) and 3 treatments with V_2, VR_{10} and VR_1 experimental biostimulants application. The biostimulants were applied by foliar spraying two times in each regrowth at 0.3 l da^{-1} application rate per treatment for all biostimulants. The first application was carried out at a plant height of 10-15 cm and the second one in alfalfa bud stage. The products applied were elaborated on the basis of 10% w/v vermicompost extracts and enrichment with an auxin-type growth regulator at the Laboratory of Biologically Active Substances to the ICFT - Sofia.

V_2 – combined vermicompost extracts with 2 extragents used, concentration 20% v/v.

VR_{10} – vermicompost extract enriched with a regulator at a concentration of 10% v/v.

VR_1 – vermicompost extract enriched with a regulator at a concentration of 1% v/v.

During the foliar fertilization, the plants on the control treatment were treated with distilled water.

To examine the effect of biostimulants were estimated the agronomical traits as follows: plants height and plant stand density, green mass yield, dry matter content in green mass and dry matter yield.

The plants height (cm) was recorded by measuring the length of the stems from the soil surface to the tip of the uppermost leaf of live shoots at 5 locations per each harvesting plot. Assessment of plant stand density, expressed by stem number (SN) per m^2 was determined by counting the stems in sampling plot (50 cm x 50 cm) at each harvesting plot for the variants. Alfalfa was harvested at early flowering stage. The green mass yield (GMY), t ha^{-1} were measured in 2019, 2020, and 2021 by harvesting of each plot and weighing in the field. A total 10 cuttings were made (2019 - two cuts, 2020 and 2021 - four cuts) during the study period. For dry matter content determination (%) 200 g fresh vegetative mass was sampled before cutting for each variant. The samples were dried to constant weight in a drying chamber at 105°C, weighed and the percentage of dry matter was estimated. Dry matter yield (DMY), t ha^{-1} was determined on the basis of green mass yield and the percentage of dry matter.

The experimental data were analysed by the One-way analysis of variance (ANOVA) and means were separated by Least Significant Differences (LSD) at 5% probability level. The statistical analysis was performed using the STATGRAPHICS PLUS software.

RESULTS AND DISCUSSION

According to the results obtained at this study, there were different effects of biostimulants application on the measured traits ($p<0.05$) in regrowths and growing seasons.

Data demonstrate that over the year of alfalfa establishment biostimulants were the most effective in first cut impacting positively stems elongation (Table 2). The reported values in second regrowth were in range from 66.40 cm in VR_{10} treatment to 62.80 cm in VR_1 at 60.12 cm in control. It was found in establishment year the real plant height at VR_{10}, VR_1 and V_2 treatments were significantly increased by 39.44%, 32.09% and 20.40%, respectively in comparison to untreated stand. The tendency outlined for positive impact of the biostimulants on the degree of phenotypic expression of the trait was kept in the first production year. The highest real plant height (average for four cuts) was ascertained at VR_{10} and VR_1 biostimulants application (60 and 59 cm, respectively) in 2020. The mean values differences both among biostimulant treatments and biostimulants and control were considerable ($p<0.05$). In contrast to the first and second experimental years, the results from the third year indicated no significant differences in real plant height. For three years the maximum mean conopy height of 64.13 cm was obtained in VR_{10} biostimulant application, followed by VR_1 treatment (62.61 cm). Analysis of variance showed that over period of investigation mean real plant height was not significantly different among VR_1 and VR_{10} treatments but was considerably higher ($p<0.05$) versus V_2 treatment (59.29 cm) and control (54.75 cm).

Table 2. Means of biostimulant treatments for grass stand height

Year	Treatment	Real plant height by cuts, cm				Mean	Difference %
		I	II	III	IV		
2019	Control	30.00	60.12	-	-	45.06 d	
	V_2	44.50	64.00	-	-	54.25 b	120.40
	VR_{10}	59.25	66.40	-	-	62.83 a	139.44
	VR_1	56.25	62.80	-	-	59.52 ab	132.09
	LSD (0.05)					5.56	
2020	Control	47.75	60.25	59.00	31.00	49.50 d	
	V_2	52.50	68.50	68.00	31.00	55.00 c	111.11
	VR_{10}	68.00	71.50	69.00	31.50	60.00 a	121.21
	VR_1	66.50	71.00	68.25	30.25	59.00 b	119.19
	LSD (0.05)					2.25	
2021	Control	92.75	75.75	83.75	26.50	69.69	
	V_2	93.00	73.75	81.25	26.50	68.63	98.48
	VR_{10}	91.75	69.75	87.50	29.25	69.56	99.81
	VR_1	95.00	74.75	83.25	24.25	69.31	99.45
	LSD (0.05)					4.64	
2019-2021	Control					54.75 c	
	V_2					59.29 b	108.30
	VR_{10}					64.13 a	117.13
	VR_1					62.61 a	114.36
	LSD (0.05)					2.77	
Annual		Average					
2019		47.50 b	63.33 b			55.42 b	
2020		58.69 b	67.81 ab	66.06 b	30.94 a	41.58 b	
2021		93.13 a	73.50 a	83.94 a	26.63 b	69.30 a	

Means followed by same letter in the columns are not significantly different at $P \leq 0.05$

The same trend was observed in other studies demonstrated that the application of biobased products positively influenced the conopy height (Marinova et al., 2019). Results of present study are in agreement with those of Getnet and Raja (2013) who reported that plant height increased significantly with addition of vermicompost. Sefaoglu et al. (2021) also reported that the application of organic and inorganic fertilizers alone and in combination created differences in the plant height in sunflower.

Data recorded on grass stand density, expressed by stems number (SN) per m^2, were present in Table 3. The means of the trait revealed that the response to the biostimulants application varied in regrowths and years. The treating alfalfa plant stands with the biostimulants not exhibited sustainable higher productivity of stems per unit area compared to the control, over the three years of study, whit exception of V_2 treatment. In the year of alfalfa establishment no significant effect of the biostimulants on the studied trait was identified; however, a tendency to increase the potential for stems development was observed after foliar spraying with biostimulants, especially at VR_{10} application in the secant cut of first year. The extent of trait phenotypic exspression in the second growing season was significantly ($p<0.05$) affected by V_2 biostimulants application. In treatment with V_2 476 stems per unit area were counted and 448 in a stand without fertilization. The treatment with VR_{10} biostimulant in 2021 had the most beneficial ffect on increasing the stems number by 8.38%, as compared to the control. The stimulating effect of the VR_{10} product was observed in all regrowths. The reported excess for V_2 was 3.86%. In turn, means for three-year of research confirmed that the highest stems number per unit area was obtained in V_2 treatment, as compared to the other biostimulants. Also, the excess of 4.78% compared to the untreated stand was statistically significant ($p<0.05$). The results were in agreement with the previous studies that foliar fertilization with some organic products contributed to development of grass stands with better density (Marinova et al., 2021).

According to a number of studies bio-based products contribute to increase the rate of photosynthesis and enhance metabolism (Popov and Dzimotudis, 2007) and positively affect the yield structural components (Churkova, 2008). On the contrary, Hall et al. (2002) in a study on the effectiveness and profitability of foliar fertilizers and growth regulators applied in alfalfa found that none of the tested foliar applied products increased stem density.

Table 3. Means of biostimulant treatments for grass stand density

Year	Treatment	Stem number per m^2 by cuts				Mean	Difference %
		I	II	III	IV		
2019	Control	164	195	-	-	179.50 ab	
	V_2	148	200	-	-	185.00 ab	103.06
	VR_{10}	148	238	-	-	193.00 a	107.52
	VR_1	144	209	-	-	176.50 b	98.33
	LSD (0.05)					13.96	
2020	Control	504	489	480	319	448.00 b	
	V_2	504	495	512	394	476.00 a	106.25
	VR_{10}	390	393	412	401	399.00 c	89.06
	VR_1	444	460	436	418	440.00 b	98.21
	LSD (0.05)					27.36	
2021	Control	434	412	391	290	381.75 b	
	V_2	438	411	389	348	396.50 ab	103.86
	VR_{10}	470	427	420	338	413.75 a	108.38
	VR_1	429	390	399	329	386.75 b	101.31
	LSD (0.05)					24.01	
2019-2021	Control					336.42 b	
	V_2					352.50 a	104.78
	VR_{10}					335.25 b	99.65
	VR_1					334.42 b	99.41
	LSD (0.05)					14.26	
Annual		Average					
2019		151.00 b	210.50 c			180.75 c	
2020		460.50 a	459.25 a	460.00 a	383.00 a	440.69 a	
2021		442.75 a	410.00 b	399.75 b	326.25 a	394.69 a	

Means followed by same letter in the columns are not significantly different at $P \leq 0.05$

Data regarding green mass yield of the alfalfa stand under different treatments both in the year of crop establishment and two production years were given in Table 4. The impact of foliarly applied products on alfalfa biomass was different over the three years of study. In the year of alfalfa establishment the use of biostimulants resulted in increase of green mass yield. The treatment of alfalfa stands with the VR_{10} biostimulant had the most

beneficial effect on increasing the fresh biomass amount. The excess of 13.26%, over the control was significant. The application of V_2 and VR_1 biostimulants increased the green mass yield by 9.86% and 3.15%, respectively, but not significantly compared to control. In 2019 no significant differences ($p<0.05$) were found between treatments including V_2, VR_1 and VR_{10} biostimulants. Results indicated that the positive effect of foliar spraying with studied biostimulants on the main economically trait was clearly expressed in first production year. It was found foliar spraying with VR_{10} significant increased yield at all regrowths, as in the establishment year. The values reported shown that total annual green mass yield varied from 111.55 t ha^{-1} at VR_1 application to 105.75 t ha^{-1} for V_2. It is evident the VR_1, V_2 and VR_{10} treatments resulted in significant ($p<0.05$) increases in yield by 20.59%, 18.11% and 14.32%, respectively compared to the control. Unlike the first and second alfalfa growing season, treatment with the biostimulants not contributed to higher green mass yield during the last production year.

Table 4. Means of biostimulant treatments for green mass yield

Year	Treatment	Green mass yield by cuts, t ha^{-1}				Total	Difference %
		I	II	III	IV		
2019	Control	5.75	6.32	-	-	12.07 b	
	V_2	6.26	7.00	-	-	13.26 ab	109.86
	VR_{10}	6.45	7.22	-	-	13.67 a	113.26
	VR_1	5.75	6.70	-	-	12.45 ab	103.15
	LSD (0.05)					1.51	
2020	Control	29.95	25.05	29.40	8.10	92.50 b	
	V_2	35.15	31.00	34.00	9.10	109.25 a	118.11
	VR_{10}	30.90	31.00	34.40	9.45	105.75 a	114.32
	VR_1	33.10	32.10	36.50	9.85	111.55 a	120.59
	LSD (0.05)					7.29	
2021	Control	35.80	33.95	28.90	2.50	101.15 a	
	V_2	36.55	29.10	24.55	1.60	91.80 b	90.76
	VR_{10}	35.10	30.40	26.70	230	94.50 b	93.43
	VR_1	35.65	27.95	24.15	250	90.25 b	89.22
	LSD (0.05)					4.55	
2019-2021	Control					68.57 b	
	V_2					71.44 a	104.18
	VR_{10}					71.31 a	103.99
	VR_1					71.42 a	104.15
	LSD (0.05)					2.29	
Annual		Average					
2019		6.05 c	6.81 b			6.43 c	
2020		32.28 b	29.79 a	33.58 a	9.13 a	26.19 a	
2021		35.78 a	30.35 a	26.08 b	2.23 b	23.61 b	

Means followed by same letter in the columns are not significantly different at $P \leq 0.05$

In terms of forage productivity for three-year study was found that application of the experimental biostimulants contributed to higher mean annual green mass yield compared to control. The summarized data shown that positive effect of V_2, VR_1 and VR_{10} biostimulants on green mass yield was equalized (by increase of 4.18, 4.15 and 3.99 %, respectively).

The same trend has been demonstrated by other authors who noted that the bio-based products increase the amount of nitrogen in the soil and support the growth of plants, resulting in higher yields (Zaman et al., 2011; Caliskan et al., 2021). Javaad and Panwar (2013) also reported the use of vermicompost alone or in combination with other organic or mineral fertilizers is effective in increasing the productivity of various plants.

Dry matter content is a characteristic which play a key role in alfalfa hay yield. Data in the present study showed the biostimulants foliar application had no effect on dry matter content in fresh biomass in year of alfalfa establishment (Table 5). In 2020 higher variability of the trait between treatments was observed. The values were in range from 26.25% to 23.75%, whit priority of the control. There was found V_2 and VR_1 application exhibited slight positive effect on dry matter accumulation in third growing season. The stimulating effect of the both products was the most clearly expressed in the first regrowth. V_2 and VR_1 application led to dry matter content increase by 5%, as compared to the control. The positive impact of the both biostimulants was kept in the second and third regrowths. Mean trait values for the three-year study shown V_2 and VR_{10} biostimulants application contributed to slight increase in dry matter content by 0.42 and 0.25 percentage units, respectively.

Table 5. Means of biostimulant treatments for dry matter content in alfaalfa biomass

Year	Treatment	Dry matter content by cuts, %				Mean	Difference +/-
		I	II	III	IV		
2019	Control	30	28	-	-	29.00	
	V_2	31	29	-	-	30.00	+ 1.00
	VR_{10}	31	30	-	-	30.50	+ 1.50
	VR_1	30	29	-	-	29.50	+ 0.50
2020	Control	25	23	30	27	26.25	
	V_2	23	21	29	26	24.75	-1.50
	VR_{10}	21	24	29	26	25.00	-1.25
	VR_1	16	23	30	26	23.75	-2.50
2021	Control	24	26	26	31	26.75	
	V_2	29	28	26	31	28.50	1.75
	VR_{10}	25	28	25	31	27.25	0.50
	VR_1	29	27	27	31	28.50	1.75
2019-2021	Control					27.33	
	V_2					27.75	0.42
	VR_{10}					27.58	0.25
	VR_1					27.25	-0.08
Annual		Average					
2019		30.50 a	29.00 a			29.75 a	
2020		21.25 b	22.75 c	29.50 a	26.25 b	24.94 b	
2021		26.75 a	27.25 b	26.00 b	31.00 a	27.75 c	

Data regarding dry matter yield for the treatments including V_2, VR_{10} and VR_1 biostimulants were one-way with those for green mass yield in the year of alfalfa establishment (Table 6). However, a more significant increases were found in DMY yield at the biostimulants application than in GMY compared to the control. VR_{10} application distinguished with stronger positive impact on annual DMY, compared to the other tested biostimulants. It can be seen that in the first production year, alfalfa DMY changed significantly among biostimulant treatment in response to the significant variation in dry matter content, mainly in the first and second regrowths. It was observed that in terms of DMY the excesses obove the control were significantly reduced in comparison the increases determined regarding GMY. This is most pronounced at VR_{10} treatment (from incease of 20.59% in GMY to 7.97% in DMY). In 2021, an opposite trend was observed. It was found that despite the relatively lower green mass yields at biostimulants foliar application, the total annual dry matter yield was equal to that of untreated variant. Over the study period the effect of V_2, VR_{10} and VR_1 biostimulants was expressed in mean annual DMY of 18.79, 18.40 and 18.35 t ha^{-1} at harvested yield of 17.90 t ha^{-1} from the untreated stand.

Our findings confirmed statements of Kavut and Avcioglu (2015) that dry matter content of forage crops is one of the dependable criteria of biomass production and high rate of dry matter content is mostly indicate a better adaptability and yield performance.

In 2019 (year of alfalfa establishment) sufficient soil moisture and high temperatures in March ensured good seed germination and normal initial development of young alfalfa plants. The significant rainfall amounts in April and May and prolonged rainy periods in June and August led to delay of growth and development of the alfalfa grass stands. In the first year of study alfalfa achieved only two cuts. In 2020 and 2021 (first and secont production year) alfalfa achieved four cuts. This explains the higher annual yields in the second and third year of study.

Data shown that the average plant height in the first and third cuts and average plant height in 2021 were statistically higher compared to those in 2019 and 2020. In second and third cuts and average for 2020 more number of stems were determined than those reported in 2019 and 2021 and differences were statistically significant.

The annual green mass yield in 2020 was statistically higher than those achieved in 2019 and 2021. Regarding dry matter yield statistically significant differences between the measured yields in 2020 and 2021 were no observed.

It can note the estimated agronomical traits was significantly influenced by year and weather conditions. Our results confirm the findings that the sustainable yields of crops are the result of the complex interaction between internal factors (variety genetic composition) (Tucak et al., 2014) and external factors (year, weather conditions, management) (Petkovic et al., 2019). According Kocira et al. (2020), effectiveness of biostimulants is determined not only the crop species, variety and environment but also by appropriate choice of preparations, their dose and method of application.

Table 6. Means of biostimulant treatments for dry matter yield

Year	Treatment	Dry matter yield by cuts, t ha^{-1}				Total	Difference, %
		I	II	III	IV		
2019	Control	1.73	1.77	-	-	3.50 c	
	V_2	1.94	2.03	-	-	3.97 ab	113.43
	VR_{10}	2.00	2.17	-	-	4.17 a	119.14
	VR_1	1.73	1.94	-	-	3.67 bc	104.86
	LSD (0.05)					442.7	
2020	Control	7.48	5.76	8.82	2.18	24.25 b	
	V_2	8.08	6.24	9.86	2.59	26.77 a	110.44
	VR_{10}	6.49	7.44	9.97	2.46	26.36 a	108.75
	VR_1	5.30	7.38	10.95	2.56	26.19 ab	107.97
	LSD (0.05)					2.08	
2021	Control	8.59	8.83	7.75	0.78	25.95 a	
	V_2	10.60	8.15	6.38	0.50	25.63 ab	98.78
	VR_{10}	8.78	8.51	6.68	0.71	24.68 b	95.12
	VR_1	10.34	7.55	6.52	0.77	25.19 ab	97.07
	LSD (0.05)					1.25	
2016-2018	Control					17.90 b	
	V_2					18.79 a	104.99
	VR_{10}					18.40 ab	102.83
	VR_1					18.35 ab	102.53
	LSD (0.05)					654.5	
Annual		Average					
2019		1.85 c	1.98 c			1.91 b	
2020		6.84 b	6.71 b	9.90 a	2.45 a	6.47 a	
2021		9.58 a	8.26 a	6.83 b	0.69 b	6.34 a	

Means followed by same letter in the columns are not significantly different at $P \leq 0.05$

CONCLUSION

The application of experimental biostimulants had a positive effect on the growth and development of alfalfa in the environmental conditions of IASS "Obraztsov chiflik". The additional introducing of VR_{10} and VR_1 biostimulants contributed to higher alfalfa grass stands. The positive impact resulted in significant increase in plant height by 17.13 and 14.36%, respectively. Foliar spraying with V_2 biostimulant had more clear pronounced positive effect on the potential for stem formation and development of grass stands with better density. According to the three-year results the average stems number per m^2 at V_2 treatment significant exceeded the control by 4.78%. V_2 biostimulant distinguished with a stronger positive effect on the GMY and DMY among studied experimental products. The mean annual GMY and DMY at treatment with the V_2 were higher by 4.18 and 4.99%, respectively compared to the control and the increases were statistically significant. Biostimulants application could be an important agrotechnical measure for increasing plant height and stem number as yield related main traits. Foliar fertilization with V_2 biostimulant gives promising results and reason to be recommended as a corrective component in technology of growing alfalfa for green forage and hay.

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